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A field assessment of the effect of pre-slaughter conditions and genetic-stress susceptibility on blood welfare indicators in pigs

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Abstract

The effect of pre-slaughter handling conditions and the RYR1 gene on blood cortisol, lactate and creatine phosphokinase (CPK) levels at exsanguination were assessed using 2,923 surveyed pigs from 106 deliveries to five Spanish abattoirs across two seasons. The relationship between blood parameters, carcase skin damage and pork quality traits was also assessed. The season influenced blood cortisol, lactate and CPK values. Females always showed higher concentrations of cortisol, lactate, and CPK than males. Pigs carrying the recessive allele of the RYR1 gene exhibited increased lactate and CPK concentrations but not cortisol. The cortisol concentration decreased in lean pigs that were slaughtered in winter after short lairage periods. The lactate concentration decreased with loading time and increased in summer with lairage time and carcase lean content. The CPK concentration increased with lairage time, carcase weight, and carcase lean content, and with the duration of winter transports. Each truck delivery only explained approximately 10% of the variance in blood parameters. Lairage time is the most influential pre-slaughter handling practice on the assessed welfare indicators. In addition, different optimal lairage times might be appropriate depending on season. Blood cortisol, lactate, and CPK concentrations increased concomitantly with skin damage score. Blood parameters were weakly correlated and they also showed low association with pork quality traits.

Keywords: *animal welfare, blood constituents, pre-slaughter, RYR1 gene, skin damage, swine*

Introduction

During the pre-slaughter and processing period, animals are exposed to a variety of handling practices in a relatively short period of time, which may cause suffering (Warriss 1996) and represent a significant source of economic loss (Guise 1991; Warriss *et al* 1998a). It is well known that stress-inducing practices such as on-farm fasting time, mixing of unfamiliar pigs at loading, loading time and lairage time lead to increased bruising and carcase damage (Guàrdia *et al* 1996, 2009) and to decreased meat quality (Faucitano *et al* 1998; Warriss *et al* 1998a; Guàrdia *et al* 2004, 2005). Stress may be caused by combining physical discomfort such as food and water restriction, fatigue due to the movements of the truck, pain due to shocks or slaps, long and rough transports, inappropriate temperatures or air speed), with psychological discomfort such as fear-induced change to the familiar situation (Terlouw *et al* 2008).

It has been shown that blood cortisol is a reliable measure of psychological stress (Knowles & Warriss 2000) whilst the concentration of lactate and creatine phosphokinase (CPK) represent good indicators of muscular activity or tissue damage (Van der Meulen *et al* 1991; Mitchell *et al* 1992; Fàbrega *et al* 2002, 2004; Yu *et al* 2007). Increased pre-slaughter stress results in higher blood cortisol and lactate, although only the latter has been shown to be strongly correlated with pork quality attributes (Barton-Gade & Christensen 1998; Hambrecht *et al* 2004, 2005). In addition, blood lactate at exsanguination has been recently proposed as a quantitative tool to improve animal handling, increased concentrations being correlated with specific pre-slaughter negative behaviours (jamming, rearing and backing up) (Edwards *et al* 2010).

In pigs, the RYR1 genotype (halothane gene) has been proven to play an important role in the response of the animal to pre-slaughter conditions (Grandin & Deesing 1998; Fisher *et al* 2000), with the recessive allele (n) being associated with stress susceptibility (porcine stress syndrome). Many studies have shown a detrimental effect of the halothane gene on both mortality rates during the pre-slaughter period (Eikelenboom *et al* 1978; Barton-Gade &

Table 1 Mean (± SD), minimum and maximum characteristics' vaues of the surveyed pigs and deliveries.

Trait[1]	Mean (± SD)	Minimum	Maximum	N
Cortisol (ng ml^{-1})	81.8 (± 30.6)	10.0	210.0	1,456
Lactate (mmol l^{-1})	13.1 (± 3.8)	1.6	27.9	2,747
CPK (log IU l^{-1})	3.83 (± 0.42)	2.58	4.84	2,671
PQM (μs)	4.33 (± 1.83)	1.10	21.00	2,761
pH_{24}	5.79 (± 0.31)	5.09	6.88	2,660
Skin damage score	2.09 (± 0.56)	1.00	5.00	2,904
Carcase weight (kg)	77.68 (± 9.77)	40.00	116.00	2,919
Carcase lean percentage (%)	56.97 (± 3.85)	38.15	66.56	2,535
On-farm fasting time (h)	14.35 (± 6.31)	0.10	34.00	2,914
Loading time (h)	1.32 (± 0.51)	0.50	3.00	2,863
Transportation time (h)	2.63 (± 1.64)	0.25	7.00	2,838
Lairage time (h)	7.06 (± 3.69)	0.83	15.00	2,923
Stocking area (m^2 100 kg^{-1})	0.39 (± 0.08)	0.24	0.79	1,820

[1] PQM (pH_{24}): electrical conductivity (pH) in the *semimembranousus* muscle at 1–2 h (24 h) post mortem.

Baltzer 1991; Fàbrega *et al* 2002; Barton-Gade *et al* 2007) and meat quality (Barton-Gade & Christensen 1998; Guàrdia *et al* 2004). However, the effect of the halothane gene on blood stress indicators is not fully understood.

The present paper has been prepared using the dataset of a survey of pre-slaughter conditions from farm loading to slaughter conducted in five Spanish abattoirs (Gispert *et al* 2000). In previous papers, we used the data from this survey to identify and assess the risk factors for pork meat becoming pale, soft and exudative (PSE) (Guàrdia *et al* 2004) and dark, firm and dry (DFD) (Guàrdia *et al* 2005), as well as for skin damage occurrence prior to slaughter (Guàrdia *et al* 2009). The current study aims at evaluating the effect of pre-slaughter handling conditions and the RYR1 genotype on blood cortisol, lactate and CPK of swine under commercial conditions in Spain. This study also explored the relationships of these welfare indicators with gender, lean content, carcase skin damage, and pork quality traits.

Materials and methods

Study animals and measurements

A total of 116 deliveries (truck loads) comprising an overall random sample of 15,695 pigs transported into five Spanish commercial abattoirs (designated as A, B, C, D and E) were surveyed during the winter (December–March) and summer (June–September) periods of the year. This study was a sample of 3,021 pigs from 106 deliveries. The characteristics of the deliveries and the pigs used in this study are summarised in Table 1.

A complete description of the abattoirs, pre-slaughter handling practices, transportation conditions and stunning methods used in the survey can be found in Gispert *et al* (2000). Briefly, the deliveries were carried out using 20 lorries and deliveries' data have been provided by the drivers after completing a questionnaire with information concerning the transport conditions. The lorries used had natural ventilation and hydraulic lifts for loading and unloading. All the pigs were mixed during lairage. A blood sample was collected at exsanguination in a randomly selected sample of approximately 20% of pigs from each from the deliveries for cortisol, creatine phosphokinase (CPK), lactate, and genotype (RYR1) determination. Immediately after collection, 4 ml of blood were separated into two eppendorf tubes. The contents of one eppendorf tube (2 ml) was put in an evacuated glass tube containing no anticoagulant for the analysis of serum cortisol and CPK, whereas the remaining one (2 ml) was put in an anticoagulant tube containing 40 μl of fluoride/EDTA solution (Boehringer, Mannheim, Germany) for the analysis of plasma lactate. The samples were centrifuged within 30 min at full speed (10,000 rpm) and then transferred to 1 ml cryotubes to be frozen in liquid nitrogen for transportation and stored at –20ºC until analysis.

Cortisol concentration was measured in 1,456 samples: 378 in A; 212 in B; 294 in C; 288 in D; and 284 in E by competitive solid-phase radioimmunoassay (Incstar Corporation, Stillwater, Minnesota, USA) and expressed as ng ml^{-1}. A mean of 13.7 pigs per journey (range 5–29 pigs) was available to determine cortisol. The serum CPK and plasma lactate concentrations were assayed by enzymatic kits (Gernon RAL, Barcelona, Spain, for CPK; and Boehringer, Mannheim, Germany, for lactate) and expressed as log IU l^{-1} and mmol l^{-1}, respectively. In the analysis of serum CPK, a dilution of the samples was necessary to guarantee the linearity of the values obtained. CPK and lactate were determined in 2,671: 701 in A; 391 in B; 579 in C; 558 in D; and 442 in E and 2,747: 748 in A; 397 in B; 586 in C; 565 in D; and 451 in E samples, respectively, with an average of

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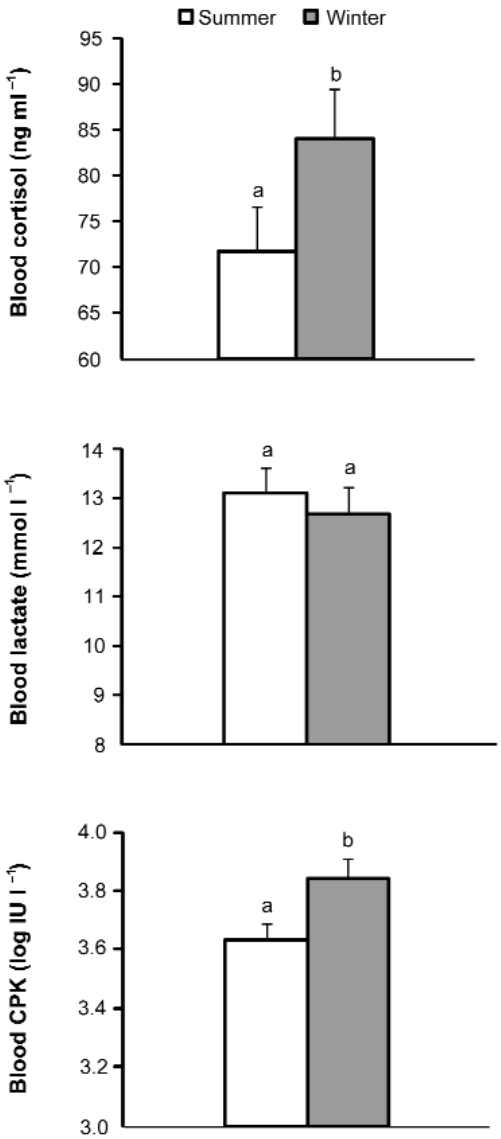
25.2 and 25.9 pigs per delivery. A minimum of six pigs and a maximum of 63 pigs per delivery were sampled to determine the concentration of both blood stress indicators. DNA was prepared from 1,244 blood samples: 349 in A; 246 in B; 235 in C; 193 in D; and 221 in E for RYR1 genotype (NN — stress resistant, Nn — carriers, and nn — stress susceptible) analysis. The HAL 1843 genotype (Inno-vations Foundation, Toronto, Canada) (homozygous nn and NN and the heterozygous Nn) was determined by PCR amplification and digestion with restriction enzymes as described previously by Fujii *et al* (1991). An average of 12.1 pigs per journey was randomly sampled from 103 deliveries.

Skin damage was assessed on the dressing line using a photographic scale (five-point scale: 1) none; 2) very slight blemish; 3) slight blemish; 4) moderate blemish; and 5) severe blemish) provided by the UK Meat and Livestock Commission (MLC 1985). Skin damage was scored as a whole carcase score. Records scored from 3 to 5 were grouped into the same category because moderate and severe blemish carcases only represented 1.6% of the total. Pork quality traits measured were electrical conductivity (PQM) and the ultimate pH (pH_{24}). PQM was measured with the Pork Quality Meter (PQM-I-INTEK, Gmbh, Aichach, Germany) and it was used to assess PSE condition. The parallel electrodes of the probe were inserted perpendicularly to the muscle fibres into the exposed surface of the *semimembranosus* muscle in the left side of the carcase at 1–2 h post mortem. Carcases showing PQM values greater than 6 µs were classified as PSE and those greater than 4 but lower or equal to 6 as prone to PSE. Meat was considered as being of normal quality when PQM was ≤ 4 µs. The pH_{24} was measured in a sample from the exposed surface of the *semimebranosus* muscle in the left side of the carcase, and it was used to assess the DFD condition. Samples were taken at 1–2 h post mortem, kept under refrigeration (3–4ºC) for 24 h, and then frozen (–20ºC). Then, pH_{24} was measured after thawing (CRISON, micropH 2001, Crison Instruments SA, Alella, Spain) in the laboratory (Solomon 1987). This pH measure involves homogenising in deionised water and measuring within 3–5 s of homogenisation. Carcases showing pH_{24} values greater than 6.2 were classified as serious DFD and those greater than 6.0 but lower or equal to 6.2 as moderate DFD. Meat was considered as being of normal quality when pH_{24} was ≤ 6.0.

Figure 1



Least square means (± SEM) of blood cortisol, lactate, and CPK concentrations by season. Different superscripts differ by $P < 0.05$.

Data analysis

Blood welfare indicators were analysed using a linear mixed model, in which fixed effects included the season (winter or summer), the abattoir (A, B, C, D, and E), the loading system (ramp or hydraulic lift) from farm to truck, the truck floor surface (polyester, aluminium, or iron), the gender (male or female and barrows) and the RYR1 genotype (nn, Nn, NN), with on-farm fasting time, loading time, transportation time, lairage time, carcase weight and carcase lean percentage as covariates. The season by abattoir interaction was significant and therefore included in the model. The interaction between every covariate and season was tested. The deliveries and the residual were the random effects. The effect of skin damage was evaluated using the same model but adding the skin damage score as a new independent variable. Before the statistical analysis, serum CPK concentration was log-transformed to achieve a normal distribution of data.

The effects and covariates were tested following the Kenward-Roger method and the differences between the least square means by the Tukey test. The proportion of the total variance due to the truck loads was used to assess the effect of the delivery. Total variance was obtained as the sum of the variance between and within (residual) deliveries. Total and partial correlations among stress indicators and meat quality traits were calculated. Partial correlations were calculated after adjusting raw data for the aforementioned pre-slaughter practices. The adjustment was conducted using a five-trait multivariate analysis with heterogeneous (co)variances. Restricted maximum likeli-

Table 2 Least square means (± SEM) of blood cortisol, lactate, and CPK concentrations by floor surface type and loading system[1]

	Cortisol (ng ml^{-1})	Lactate (mmol l^{-1})	CPK (log IU l^{-1})
Floor surface			
Polyester	80.1 (± 5.1)	12.5 (± 0.5)	3.71 (± 0.06)
Aluminium	76.7 (± 6.9)	13.3 (± 0.7)	3.75 (± 0.09)
Iron	82.6 (± 7.0)	12.1 (± 0.8)	3.78 (± 0.09)
Loading system			
Hydraulic lift	80.3 (± 9.7)	12.3 (± 1.1)	3.67 (± 0.12)
Ramp	79.4 (± 2.5)	13.0 (± 0.3)	3.83 (± 0.03)

[1] Within each column and effect, all differences are non significant ($P > 0.05$).

hood estimators were used for variances and covariances. The threshold for statistical significance was set at $P < 0.05$. The analyses were carried out using SAS PROC MIXED (SAS Institute Inc, Cary, NC, USA).

Results and discussion

Effect of season

Blood cortisol and CPK but not lactate differed by season (Figure 1). On average, the concentration of cortisol and CPK was higher in winter (17.3 and 5.8%, respectively), which might suggest that pigs were more stressed in winter than in summer. The unfavourable effect of winter could be partly related to the few abattoirs analysed. Accordingly, this effect was only found significant for abattoirs C and E, for cortisol, and for abattoirs B and E, for CPK.

Regarding cortisol concentrations, the current results are in accordance with those of Baldwin and Stephens (1973) and Averós *et al* (2007) who recorded higher cortisol secretion under cold temperature, which was attributed to higher energy demand to maintain the body temperature. Cortisol is the major adrenocortical hormone secreted in response to the release by the pituitary gland of adrenocorticotrophic hormone (ACTH). In stressful situations, cortisol release results in an elevated plasma glucose concentration through increased hepatic glycogenolysis and gluconeogenesis coupled with increased protein catabolism (Shaw & Tume 1992). In hot temperatures, pigs lie down (Hicks *et al* 1998; Geers 2007) and were less likely to engage in aggressive behaviour (Morrow-Tesch *et al* 1994; Hicks *et al* 1998). Consequently, it would be expected that pigs were less active in summer and consequently they have lower cortisol and CPK concentrations.

Effect of pre-slaughter handling

No significant effect of the flooring of the truck or the loading system was found on blood welfare indicators (Table 2). Guàrdia *et al* (2004, 2005, 2009) observed a significant effect of the type of flooring on the risk of incidence of PSE, DFD and skin damage. However, the results obtained in the current work failed to significantly demonstrate a persistent effect of truck flooring and loading system on physiological indices of stress, in line with results obtained by Nanni Costa *et al* (1999). Brown *et al* (2005) observed that differences during loading in heart rate between the ramp and the hydraulic tail-lift systems were small but dependent on the slope of the ramp. According to Warriss *et al* (1991), if the ramp slope is less than 20º, there should be no negative consequences on animal handling and meat quality. The slope of the ramps used in the present study was not measured but it was likely < 20º, a circumstance that may have contributed to weaken the differences among flooring types and loading devices.

Table 3 shows the variation in cortisol, lactate and CPK concentrations per 1 h increase of on-farm fasting, loading, transport and lairage time by season. This value reflects the influence of each covariate on the blood parameters assessed. No effect of on-farm fasting time on blood indicators was found ($P > 0.05$). However, an effect of the loading time on lactate concentrations was observed ($P < 0.05$). Loading is known to be the most stressful phase of transport (Warriss *et al* 1998b). In recent years, some studies have shown that a short loading time involves worse handling practices because the equipment used at loading is generally not good enough to allow a fast access onto the truck without inducing additional stress (McGlone *et al* 2004; Correa *et al* 2010). Thus, our results, which showed that there was a decrease of nearly 1 mmol l^{-1} in lactate for each additional hour at winter loadings, would support that fast loadings, particularly in cold environments, lead to increased physical exercise and stress. This is also in accordance with Guàrdia *et al* (2004), who found that short loading times increase the risk of PSE occurrence. Breakdown of muscle glycogen, as a result of extreme muscular exertion, can lead to the production of large quantities of lactate which will be released into the bloodstream. Hence, elevated blood lactate concentrations may follow from excessive muscular activity. Additionally, catecholamine release as a result of fear or excitement can also lead to rapid glycogenolysis and thus excessive lactate production (Shaw & Tume 1992).

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Table 3 Variation (± SEM) in blood cortisol, lactate and CPK concentrations per 1 h increase of on-farm fasting, loading, transport, and lairage time by season.

	Cortisol (ng ml^{-1})	Lactate (mmol l^{-1})	CPK (log IU l^{-1})
On-farm fasting time			
Summer	0.4 (± 0.6)	0.00 (± 0.07)	–0.012 (± 0.008)
Winter	–0.3 (± 0.3)	–0.03 (± 0.03)	–0.004 (± 0.004)
Loading time			
Summer	–5.4 (± 6.6)	–0.70 (± 0.71)	–0.160 (± 0.084)
Winter	3.3 (± 3.4)	–0.99 (± 0.36)**	–0.019 (± 0.043)
Transportation time			
Summer	0.0 (± 2.3)	0.10 (± 0.25)	–0.009 (± 0.029)
Winter	–0.7 (± 1.4)	0.20 (± 0.15)	0.047 (± 0.018)**
Lairage time			
Summer	0.8 (± 0.7)	0.17 (± 0.08)*	0.024 (± 0.009)*
Winter	–1.6 (± 0.6)**	–0.06 (± 0.06)	0.029 (± 0.007)**

* $P < 0.05$; ** $P < 0.01$. Values without asterisks did not differ significantly ($P > 0.05$).

In the present study, as in that reported by Honkavaara (1989), transport time resulted in an increase in CPK concentrations in winter (0.047 log IU l^{-1} h^{-1}). Pigs need more energy to maintain body temperature during winter (Baldwin & Stephens 1973), and hence long winter transports may be a source of physical stress. This trait may be linked to the higher risk of DFD pork occurrence in winter (Guàrdia *et al* 2005), since greater stress tends to be reflected in more DFD meat (Warriss *et al* 1998a).

Transportation time did not affect blood cortisol and lactate concentrations. Warriss *et al* (1998b) observed that pigs transported from farms over a long distance had higher CPK and cortisol but lower lactate concentrations. On the contrary, Pérez *et al* (2002a) found that cortisol and lactate concentrations were higher in pigs transported for 15 min than in those transported for 3 h. Moreover, the stocking density during transportation did not significantly affect blood cortisol ($P = 0.87$), lactate ($P = 0.64$), and CPK ($P = 0.53$) concentrations and therefore was not included in the final models.

Lairage time was the variable that most influenced animal blood stress indicators in the present work (Table 3). A longer time spent in lairage decreased cortisol concentrations in winter (1.6 ng ml^{-1} h^{-1}) and increased lactate concentrations in summer (0.17 mmol l^{-1} h^{-1}). CPK increased with lairage time in both seasons (0.024 log IU l^{-1} and 0.028 log IU l^{-1} h^{-1}, respectively). The aim of lairage is, first, to allow animals to recover from transport and second, to provide a reservoir of animals for the slaughter line (Warriss 2003). Loading, transport and unloading can be traumatic for the animals and a period in lairage could allow some recovery from previous stressful handling. For this reason, appropriate lairage conditions could reduce the incidence of PSE meat (Fortin 1989; Warriss *et al* 1998b). However, it could also increase skin damage and the risk of DFD pork because of aggressive behaviours among unfamiliar pigs and/or decrease in glycogen stores due to long fasting periods (Warriss *et al* 1998b; Warriss 2003; Guàrdia *et al* 2005). According to the current results, the effect of lairage time on blood cortisol and lactate may be interacting with ambient temperature, which induces a differential stress response in each season.

The cortisol data from the present study provide evidence that the pigs are indeed experiencing lower stress response by increasing lairage time during winter. This finding might be used to reduce the aforementioned increase in blood cortisol throughout the winter season. The increase of CPK as lairage time increases indicates that long lairage periods favour physical exercise, which may result in physical stress and muscle damage (Warriss *et al* 1998c). Assuming the role of blood cortisol as an index of physiological stress and lactacte and CPK as indexes of physical stress (Fàbrega *et al* 2002; Warriss 2003), the present results showed that, particularly in winter, longer periods of lairage may decrease psychological stress due to handling and novelty but may increase physical stress due to fatigue. Several authors have proposed that no lairage or a long lairage period (> 3 h) without food may compromise animal welfare and meat quality (Pérez *et al* 2002b), suggesting that optimum lairage time may range from 1 to 3 h. Average lairage time in the present study was 7.0 h, so it is yet possible to control the length of lairage in order to reduce its negative impact on the ante mortem animal welfare.

Effect of gender, carcase weight and lean percentage

Cortisol, lactate and CPK concentrations were greater in females than in males (Table 4), in line with the results obtained by other authors (Van der Wal *et al* 1999; Pérez *et al* 2002a; Averós *et al* 2007). Males have more energy reserves in their muscles and they are more accustomed to chronic

Table 4 Least square means (± SEM) of blood cortisol, lactate and CPK concentrations by gender and RYR1 genotype.

	Cortisol (ng ml^{-1})	Lactate (mmol l^{-1})	CPK (log IU l^{-1})
Gender			
Female	83.3 (± 4.9)[a]	12.99 (± 0.53)[a]	3.77 (± 0.06)[a]
Male	76.4 (± 5.0)[b]	12.27 (± 0.54)[b]	3.72 (± 0.06)[b]
Genotype[1]			
nn	78.3 (± 7.3)	13.77 (± 0.76)[a]	4.14 (± 0.08)[a]
Nn	84.9 (± 5.4)	12.73 (± 0.63)[a]	3.86 (± 0.07)[b]
NN	79.1 (± 5.3)	12.02 (± 0.62)[b]	3.57 (± 0.06)[c]

[1] RYR1 genotype: NN: homozygous normal; Nn: heterozygous carrier; nn: homozygous carrier.
[a, b] Within each column and effect, means with different superscript differ significantly ($P < 0.05$).

Table 5 Variation (± SEM) in blood cortisol, lactate and CPK concentrations per unit increase of carcase weight and lean percentage by season.

	Cortisol (ng ml^{-1})	Lactate (mmol l^{-1})	CPK (log IU l^{-1})
Carcase weight			
Summer	0.2 (± 0.2)	0.03 (± 0.02)*	0.008 (± 0.002)**
Winter	0.2 (± 0.1)	–0.01 (± 0.01)	0.004 (± 0.001)**
Carcase lean percentage			
Summer	–0.2 (± 0.4)	0.11 (± 0.03)**	0.022 (± 0.004)**
Winter	–0.7 (± 0.3)*	0.09 (± 0.03)**	0.011 (± 0.003)**

* $P < 0.05$; ** $P < 0.01$. Values without asterisks do not differ significantly ($P > 0.05$).

stress as a result of their more aggressive sexual behaviour (van der Wal *et al* 1999). Guàrdia *et al* (2005), using the same surveyed data, observed that females and castrates were more prone to produce DFD pork than intact males. This matches data from the literature in which boar meat quality scores are better than those of gilts (Walstra *et al* 1971).

Blood lactate and CPK concentrations increased with carcase weight and lean percentage (Table 5). Our results highlighted that heavy pigs are more demanding in energy use than light pigs, in accordance with Elbers (1991), Fàbrega *et al* (2002) and Terlouw and Rybarczyk (2008), who observed that a higher bodyweight may increase energy use and hence muscle lactate breakdown during pre-slaughter walking. We also found that cortisol concentrations decreased with carcase lean percentage, particularly in winter, but it was not affected by carcase weight. This result is in accordance with the general statement that fatter breeds, like Meishan or Duroc, produce more cortisol (Mormède *et al* 2004). Foury *et al* (2005) observed that carcase lean content and cortisol concentration were negatively correlated, which confirms the positive metabolic effect of circulating cortisol on fat accretion.

The results of the present work suggest that females are more sensitive to physical stress (increased muscular activity) than males but not necessarily to psychological stress (increased glucocorticoid secretion). At similar bodyweight, the greater concentrations of cortisol in females might be related to increased adiposity compared to males. In addition, results on lactate and CPK may suggest that heavier and leaner pigs are more prone to physical stress than lighter and fatter ones, regardless of gender.

Effect of RYR1 genotype

The allele n of the RYR1 gene causing porcine stress syndrome is the main cause of PSE pork in pigs subjected to acute stress before slaughter (Cassens *et al* 1975). It can also lead to high ante mortem mortality rates (Murray & Johnson 1998). Our results showed that nn pigs had higher concentrations of lactate and CPK but not cortisol (Table 4), with Nn pigs displaying intermediate results as compared to NN pigs. No significant deviation of the heterozygote from the average of the two homozygotes was found for lactate and CPK concentrations ($P = 0.56$ and $P = 0.84$, respectively), which provides evidence that the allele n, with respect to physical stress susceptibility, acts additively. Some investigators have found that nn pigs, and to lesser extent Nn pigs, are unusually responsive to new environment stimuli, being more prone to catecholamine overloading and higher heart rates (Gregory & Wotton 1981) and present higher CPK concentrations (Rundgren *et al* 1990; Gispert *et al* 2000; Fàbrega *et al* 2004) after stressful circumstances like pre-slaughter practices. However, animals carrying the allele n of the RYR1 gene causing

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porcine stress syndrome have displayed greater blood cortisol than their resistant counterparts in some experiments (Fàbrega *et al* 2002; Pérez *et al* 2002b) but not in others (Gispert *et al* 2000; Fàbrega *et al* 2004). Porcine stress syndrome was initially detected in pigs by exposure to halothane gas, but it was established (Fujii *et al* 1991) that the condition was due to a recessive mutation of the RYR1 gene, which refers to the ryanodine receptor that regulates the release of Ca^{2+} in skeletal muscle. Collectively, the greater blood lactate and CPK in carrier pigs (nn and Nn) confirms the relationship of genetic stress susceptibility with impaired muscular function, whereas glucocorticoid secretion would not be fully dependent on muscle damage.

The effect of the RYR1 gene on physiology and behaviour has been investigated less than its effect on carcase and meat quality. It is known that there is a clear relationship between RYR1 genotypes and carcase lean percentage, with Nn and nn pigs producing leaner carcases (Tor *et al* 2001). Interestingly, after including the effect of RYR1 genotype, CPK still showed a small positive association with lean content, particularly in summer (0.009 [± 0.005], $P = 0.04$). However, lactate showed no association to lean content after this adjustment ($P = 0.15$). This result indicates that the effect of carcase lean content on physical stress markers is mainly exerted through its relationship with the RYR1 gene and therefore leanness cannot be considered as an inherent risk factor, except in stressful situations occurring in summer.

Effect of skin damage score

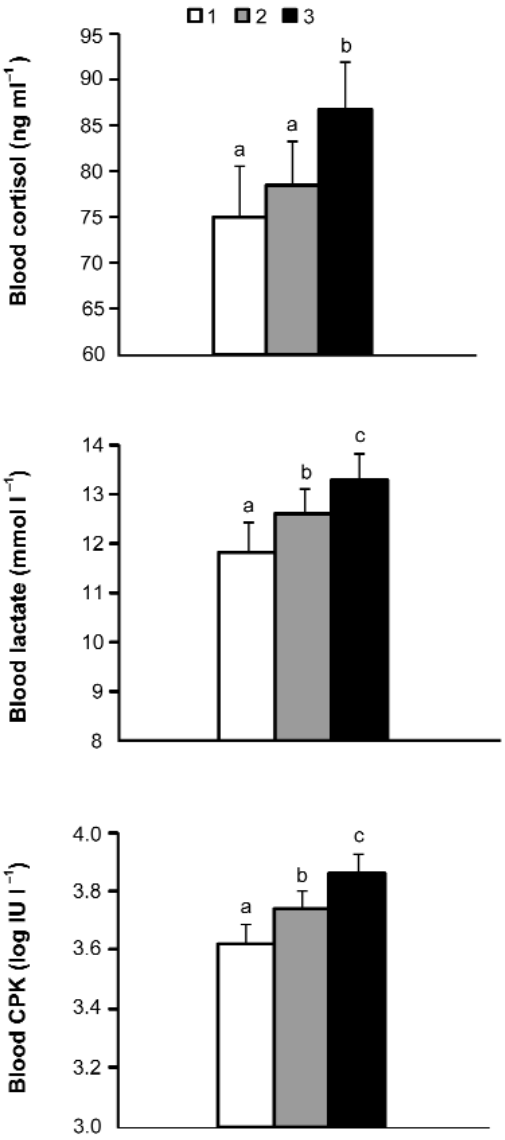
A relationship was found between skin damage score and the concentrations of cortisol, lactate and CPK (Figure 2). Increased concentrations of blood cortisol, lactate, and CPK (+11.8 ng ml^{-1}, +1.45 mmol l^{-1}, and +0.24 log IU l^{-1}, respectively) were observed in pigs producing slightly damaged carcases (score 3) when compared to pigs whose carcases were not skin damaged (score 1).

Warriss *et al* (1998a) showed that animals with more skin blemishes had higher blood cortisol and CPK concentrations, as well as a trend to increased lactate concentrations. Barton-Gade and Christensen (1998) also found that lactate and cortisol concentrations were associated with the skin blemish score. A similar trend in physiological indices of stress was recently observed by D'Eath *et al* (2010), but these authors failed to associate high numbers of skin lesions on the carcase with meat quality measures. The link between blood parameters and skin damage scores suggests that this measure may be a useful trait to mirror aggression between pigs and/or poor handling/facilities that could result in injury.

The effect of delivery

Table 6 shows the variance between deliveries in relation to the total variance for welfare blood indicators and meat quality traits. Variance between deliveries for blood indicators accounted for approximately 11% of the total variance, which was nearly two-fold the value observed for quality traits (PQM and pH_{24}) (approximately 7%). This means that the particular conditions under which the delivery is done affect more blood welfare than pork quality indicators but also that around 90% of the variation associated to pig blood stress indicators still remain unknown.

Figure 2



Least square means (± SEM) of blood cortisol, lactate and CPK levels by skin damage score: 1) none; 2) very slight blemish; and 3) slight to severe blemish. Different superscripts differ by $P < 0.05$.

Correlations between blood stress indicators and meat quality traits

The correlations among blood stress indicators, as well as the correlation between blood stress indicators and meat quality traits are shown in Table 6. Welfare blood markers, such as cortisol, lactate and CPK, are triggered upward in stressful situations, whereas there is a decline in meat quality traits indicated by increased PQM values and pH_{24}. Our results revealed that lactate had a greater correlation with PQM values ($r = 0.24$) than with pH_{24} ($r = 0.16$) whereas CPK had a greater correlation with pH_{24} ($r = 0.22$) than with PQM values ($r = 0.02$). The fact that PQM values and pH_{24} were not correlated indicates that two independent phenomena lie behind these different measures. It

Table 6 Variance between deliveries as a proportion of the total variance for blood welfare and meat quality indicators (on diagonal) and partial (above diagonal) and total (below diagonal) correlations among them[1].

	Cortisol	Lactate	CPK	PQM	pH_{24}
Cortisol	**0.10**	0.15	0.18	0.06	0.02^{ns}
Lactate	0.23	**0.11**	0.14	0.24	0.16
CPK	0.23	0.15	**0.12**	0.02^{ns}	0.22
PQM	0.00^{ns}	0.18	0.14	**0.06**	-0.03^{ns}
pH_{24}	0.17	0.14	0.26	−0.05	**0.07**

[1] All correlations are significant at $P < 0.05$ except those marked as not significant (ns). Partial correlations were calculated after adjusting raw data for pre-slaughter practices.

has long been known that meat quality is strongly influenced by the behavioural and physiological status of the animals before slaughter (Lawrie 1966). Physical activity diminishes muscle glycogen reserves. Increased physical activity coupled with long-term stress conditions during the ante mortem period causes animals to use up all their energy and gives rise to higher pH_{24} and, consequently, DFD meat condition incidence. Likewise, increased physical activity and stress immediately prior to slaughter when the pigs have sufficient reserves of energy to cause a rapid increase in lactic acid content during the immediate post mortem period has been correlated with a faster pH decline, and high incidence of PSE meat condition. Warriss *et al* (1998a) were not able to find a relationship between blood stress indicators and PSE meat characteristics, although they observed that longer stress situations tended to be reflected in more DFD meat. Nevertheless, D'Eath *et al* (2010) found that high blood lactate was associated with high drip loss, while high cortisol and CPK were associated with high pH_{24} and changes in meat colour. In general, partial correlations were lower than total correlations, particularly among blood indicators (Table 6). This result would indicate that pre-slaughter practices are partly affecting blood parameters concomitantly. Similarly, the correlation between cortisol and pH_{24}, as well as that between CPK and PQM values, vanished after adjusting for pre-slaughter conditions. Consistent with the results mentioned above on the effect of RYR1 genotype, the partial correlation between lactate and PQM values ($r = 0.24$) decreased to a very low value ($r = 0.08$, $P < 0.05$) when additionally adjusted for the RYR1 genotype.

Animal welfare implications and conclusion

There is evidence that both pre-slaughter conditions and the RYR1 genotype influence blood cortisol, lactate, and CPK. Although not in all abattoirs, conditions for pig welfare were worse in winter than in summer. Lairage time is the most influential pre-slaughter handling practice on pig blood welfare indicators. In summer, as lairage time increases, blood lactate and CPK increase, while in winter, cortisol decreases and CPK increases. Different optimal lairage times might be appropriate depending on the season. A good strategy to decrease physical stress and DFD occurrence may be therefore to decrease the lairage time. In winter, as loading time increases, lactate concentrations decrease. Therefore, regarding pre-slaughter management, more relaxed loadings will reduce physical stress and the risk of obtaining PSE meat. Considering blood lactate and CPK as muscular activity markers, the results suggest that females are more sensitive to physical stress than males. The allele n of the RYR1 gene displays an additive behaviour for lactate and CPK concentrations, indicating that heterozygotes are more prone to physical stress than stress-resistant homozygotes. The unfavourable effect of carcase lean content on physical stress markers is mainly exerted through the RYR1 gene. It is yet possible to improve pig welfare and pork quality in each truck delivery under commercial conditions. Increased concentrations of blood welfare indicators were associated with both damaged carcases and reduced pork quality. Therefore, monitoring skin damage may help to test practical ante mortem handling practices and, as a result, improve pig welfare.

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